

# Nutritional Deficits During Early Development Affect Hippocampal Structure and Spatial Memory Later in Life

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Development rates vary among individuals, often as a result of direct competition for food. Survival of young might depend on their learning abilities, but it remains unclear whether learning abilities are affected by nutrition during development. The authors demonstrated that compared with controls, 1-year-old Western scrub jays (*Aphelocoma californica*) that experienced nutritional deficits during early posthatching development had smaller hippocampi with fewer neurons and performed worse in a cache recovery task and in a spatial version of an associative learning task. In contrast, performance of nutritionally deprived birds was similar to that of controls in 2 color versions of an associative learning task. These findings suggest that nutritional deficits during early development have long-term consequences for hippocampal structure and spatial memory, which, in turn, are likely to have a strong impact on animals' future fitness.

*Keywords:* spatial memory, hippocampus, development, nutrition, *Aphelocoma californica*

Most avian species demonstrate significant individual variation in development rates within populations and among siblings within family broods, but the fitness consequences of such variation are unclear (O'Connor, 1984; Ricklefs, 1983). Whereas genetic factors might explain some of the variation, most differences in growth rates appear to be due to food availability (O'Connor, 1984; Ricklefs, 1983). Altricial birds grow very rapidly and depend entirely on their parents for food until they learn to forage independently (O'Connor, 1984; Ricklefs, 1983). Young birds suffer high mortality after they become independent, and the slower growing ones often show the lowest survival rates (Merilla & Wiggins, 1995; O'Connor, 1984; Ricklefs, 1983). The causes of high mortality in 1st-year animals are not well understood, but it has been suggested that learning abilities of young play a critical role in their survival, and thus selection pressure on learning could be high (Dukas, 1998; Weathers & Sullivan, 1989). It has been well documented that song learning in birds is negatively affected by nutritional deprivation during early posthatching development (Buchanan, Spencer, Goldsmith, & Catchpole, 2003; Nowicki,

Searcy, & Peters, 2002; Spencer, Buchanan, Goldsmith, & Catchpole, 2003). It is less known, however, whether other learning abilities are also dependent on nutritional condition and development rates during the early posthatching period or whether slower growing individuals might suffer higher mortality specifically because nutritional deficits cause learning impairments later in life.

In mammals, the effects of malnutrition on the development of the brain and cognitive abilities have received considerable attention (Bedi, 2003; Dauncey & Bicknell, 1999). It has been suggested that malnutrition during early postnatal development might have a negative impact on the maturation of the brain and the hippocampus in particular (Bedi, 2003; Dauncey & Bicknell, 1999). Because the hippocampus has been implicated in mediating spatial memory in both mammals and birds (Sherry, Jacobs, & Gaulin, 1992), it is logical to predict that malnutrition during development might negatively influence spatial memory later in life (Bedi, 1992; Bedi, 2003; Dauncey & Bicknell, 1999).

Birds exhibit tremendous variation in growth rates within broods, but differences in body mass largely disappear after young become independent (O'Connor, 1984; Ricklefs, 1983). Thus, to understand possible fitness consequences of such variation, it is crucial to determine whether nutritional rehabilitation after independence is established can alleviate the effects of early malnutrition caused by insufficient parental care and whether nutritional deficits imposed by parents on their young during the early posthatching period have long-term negative consequences that cannot be overcome by abundant feeding later on.

In the current study, we evaluated experimentally whether nutritional deficits within a range commonly observed in wild birds during early posthatching development might affect hippocampal structure and spatial memory function in Western scrub jays (*Aphelocoma californica*) long after they become nutritionally independent. The ability to recover food caches, which is largely dependent on spatial memory (Shettleworth, 1995), is a crucial factor affecting survival in food-caching birds (Pravosudov & Lucas,

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2001) and thus impaired memory ability might have a dramatic impact on the future fitness of these animals.

## Method

### Subjects

Twenty-four Western scrub jays (*Aphelocoma californica*) were taken from 12 nests at approximately 7 days of age during April 2003. Two chicks were collected from each nest and randomly assigned to the nutritionally deprived or control group: initial body mass across all birds,  $t(22) = 0.04$ ,  $p = .96$ , or within pairs,  $t(11) = 0.08$ ,  $p = .93$ . We fed chicks with Roudy Bush Hand Feed Formula 3 using plastic syringes, which allowed precise measurements of the amount fed to each individual. Control chicks were fed until satiation, whereas nutritionally deprived chicks received 65% of the amount of food given to the control chicks at each feeding. Hand feeding continued until all chicks started eating on their own and completely refused our feeding attempts. Once jays became nutritionally independent they were given food ad libitum (peanuts, Roudy Bush pellets, waxworms, and mealworms). Birds were maintained individually in cages (120 cm  $\times$  42 cm  $\times$  60 cm) on a natural photoperiod until the beginning of testing, after which they were placed on a winter-like photoperiod (9–15-hr light–dark cycle).

### Experimental Room

Jays were tested individually in the experimental room (325  $\times$  218  $\times$  312 cm), and their behavior was observed and videotaped through a one-way Plexiglas window. The room contained a rectangular board (185  $\times$  64 cm) elevated 120 cm above the floor level. The board contained 38 potential cache sites (28-mm deep holes, 55 mm in diameter), evenly distributed, and a feeder was located exactly in the middle of the board. All holes were filled with sand in which jays could cache food. A perch was attached to the long edge of the board and elevated about 10 cm so that a bird sitting on the perch had a full view of the board. All birds were familiarized with the experimental room prior to behavioral testing.

### Cache-Recovery Task

The cache recovery task consisted of a caching phase and a recovery phase separated by a retention interval of either 1 day or 10 days. Each bird was deprived of food for 24 hr before caching. During the caching phase, birds were given 30 waxworms and 20 food pellets (Roudy Bush) and were allowed to eat and cache for 40 min, after which they were placed back in their cages with unlimited access to food. We recorded all sites in which birds cached food and the content of each site. Prior to the recovery phase, birds were deprived of food 2 hr before lights went off in the evening and were then tested the next day. Before the recovery phase, we replaced all the caches made by the animal in their original locations, covered them with fresh sand, and evened the surface of the sand so that the bird could not use any local information to find its caches. The recovery phase lasted 20 min, and the only food available was that previously cached by the animal. Every time a bird probed the sand with its bill at one of the available sites, the action was recorded as a search. The cache recovery task started when birds were approximately 6.5 months old.

### Associative Learning Task

An associative learning task consisted of two phases. During Phase 1, birds were allowed to locate a single hole filled with mealworms instead of sand while all other holes were filled with sand. All birds were deprived of food 24 hr prior to Phase 1, and Phase 1 lasted 15 min or until a bird ate one mealworm (which all birds did). The individual was then removed from the experimental room for a 2-hr retention interval, after which it was tested in Phase 2. During Phase 2, food was placed at the same site where

the bird had previously found food. All potential cache sites were filled with fresh sand so that the bird could not use any local information but had to rely on its memory to find the food (see below for the two versions of the task). Every time a bird probed sand in a hole, the action was recorded as a search. If a bird probed the same hole several times, it was recorded as a single search unless the bird probed a different hole in between, in which case it was recorded as a different search. The bird was rewarded for successful search with a worm, which was placed in the hole prior to Phase 2 and which the bird could eat once it inspected the correct location.

During the spatial version of an associative learning task, birds had to rely on spatial information only, as all sites were uniform in color and shape. We ran five rewarded trials (one trial every 5th day) of the spatial version, during which the location of the food remained the same. Five days after the fifth rewarded trial, we ran an unrewarded probe trial, which consisted of Phase 2 only. If the birds remembered that food was always at the same location during the previous five trials, they would be expected to look for food in that location. The unrewarded probe also served as a control for the use of olfactory cues. The spatial version of the task started when birds were approximately 8 months old.

During the first color version of an associative learning task, a single new location containing food was marked with a unique combination of red and blue tape so that the birds could rely on both spatial and local (color) information to find the food. We ran five rewarded trials during which the food was placed at the same location marked by local cues, one trial every 5th day. Five days after the fifth trial, we ran an unrewarded color dissociation probe trial. This probe consisted of Phase 2 only, and the color pattern associated with food during the first five trials was moved to a new location (21 cm away) in order to test whether the birds relied preferentially on spatial or color information to find the food location. The color version of the task started when birds were approximately 9.5 months old.

During the second color version of an associative learning task, food was associated with one of five available colors. The spatial locations of all five colors were changed pseudo-randomly between Phases 1 and 2 as well as between trials. Thus, birds had to learn that the food was associated with a specific color, irrespective of its spatial location. We ran four rewarded trials every 5th day. Five days after the fourth trial we ran an unrewarded probe trial in which all colors were associated with uniquely new spatial locations.

### Brain Measurements

All birds were sacrificed at approximately 12 months of age to evaluate possible changes in brain morphology. Jays were anesthetized with Nembutal-sodium solution and perfused transcardially with 4% paraformaldehyde in phosphate buffer. After perfusion, birds were decapitated and their brain was removed from the skull and placed in 4% paraformaldehyde for 1 week. The brains were cryoprotected in a 30% sucrose solution, frozen in dry ice, and kept at  $-70^{\circ}\text{C}$  until processing. We cut coronal sections at 40  $\mu\text{m}$  on a sliding freezing microtome. All detailed of perfusions and brain preparations have been described in detail previously (Pravosudov & Clayton, 2002; Pravosudov, Lavenex, & Clayton, 2002; Pravosudov & Omanska 2005a, 2005b).

We used StereoInvestigator software (Version 3.15a; Microbrightfield, Colchester, VT) for all stereological measurements. We followed the Cavalieri principle in measuring the volume of the right and left sides of the hippocampus and the rest of the telencephalon on Nissl-stained sections. To measure hippocampal volume, we used 350- $\mu\text{m}$  grid size, and we measured every 20th section (15–17 per bird), which were 800  $\mu\text{m}$  apart. For telencephalon measurements we used 1,538.5  $\mu\text{m}$  grid, and we also measured every 20th section (22–24 sections per bird), which were 800  $\mu\text{m}$  apart. We used the optical fractionator method (40  $\mu\text{m}$   $\times$  40  $\mu\text{m}$  counting frame with a 400  $\mu\text{m}$   $\times$  400  $\mu\text{m}$  scan grid and a 5  $\mu\text{m}$ -high disector) to estimate the total number of neurons on the right and left sides of the hippocampus using the same sections as for the analyses of the hippocampal volume. The details of all these methods have been described previ-

ously (Pravosudov & Clayton, 2002; Pravosudov et al., 2002; Pravosudov & Omanska 2005a, 2005b).

In statistical analyses of the hippocampus we used telencephalon volume and the length of ulna as covariates to control for the variance due to different size of birds. To compare relative telencephalon volume, we used ulna length as a covariate.

## Results

### Development

Nutritionally deprived birds were observed eating food on their own for the first time at  $32.6 \pm 3.5$  SD days of age, about 3 days later than controls ( $29.4 \pm 2.4$  days),  $t(22) = -2.56, p = .02$ , but they continued to depend on hand feeding until they were  $74.0 \pm 3.5$  SD days old, about 19 days older than controls ( $55.1 \pm 12.1$  days),  $t(20) = -3.99, p < .001$ , when they reached full nutritional independence. Birds in both groups started caching on the same day they started eating on their own.

Birds that were nutritionally deprived during the early post-hatching period were approximately 24% lighter than controls at about 30 days of age,  $t(20) = -9.79, p < .001$  (see Figure 1), when they still depended on hand feeding. From the beginning of treatment until the birds reached 30 days of age, the differences in body mass between nutritionally deprived and control scrub jays varied from 10.6% to 29.4% (mean = 22.7%), which is similar to natural variation in wild Western scrub jays (difference between the smallest and largest nestlings for any given age = 37.8%, range = 6.5%–68.7%; Ritter, 1984) and in Florida scrub jays, *Aphelocoma coerulescens* (mean = 38.5%, range = 9.5%–60.4%; Woolfenden, 1978). At approximately 76 days of age, after the birds became nutritionally independent and birds in both groups were given food ad libitum, the group differences in mass were no longer significant,  $t(20) = -1.64, p = .12$  (Figure 1), and they remained nonsignificant throughout the duration of the experiment ( $p > .10$ ). Structurally, however, nutritionally deprived jays remained slightly (approximately 3%) smaller than controls when measured at 12 months of age: ulna length,  $t(20) = -2.54, p = .02$ . Thus, although nutritionally deprived birds largely recovered physically, as indicated by body mass, the recovery was not complete.

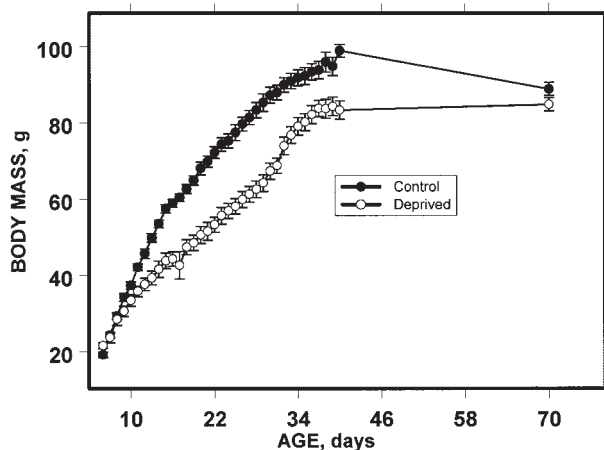


Figure 1. Body mass of nutritionally deprived (open circles) and control (filled circles) scrub jays during the first 70 days posthatching.

### Cache-Recovery Task

First, we compared memory performance of approximately 6.5-month-old nutritionally deprived and control birds in a cache-recovery task in which they were allowed to cache food and retrieve it either 1 or 10 days later. There were no significant differences between the groups in the amount of food items cached,  $F(1, 18) = 0.57, p = .46$  (see Figure 2), or in the number of sites used for caching,  $F(1, 18) = 0.60, p = .45$  (see Figure 2), suggesting that motor behavior and motivation to cache were not affected by nutritional deficits during development. Nor did repeated measures GLM indicate significant differences among the four trials (three with a 1-day retention interval and one with a 10-day retention interval) in the number of caches: trial,  $F(1, 54) = 2.59, p = .06$  and Treatment  $\times$  Trial,  $F(3, 54) = 1.46, p = .24$ . Similarly, no differences among the trials were evident in the number of caching sites used: trial,  $F(3, 54) = 1.05, p = .38$ , and Treatment  $\times$  Trial,  $F(3, 54) = 1.72, p = .24$ . Compared with control birds, however, nutritionally deprived birds inspected significantly more locations in order to find their caches after either a 1-day or a 10-day retention interval,  $F(1, 18) = 31.32, p < .001$  (see Figure 3), suggesting impaired spatial memory performance. Performance of birds in both groups did not differ significantly among the trials with a 1-day retention interval and the trial with a 10-day retention interval: trial,  $F(3, 54) = 0.32, p = .81$ , and Treatment  $\times$  Trial,  $F(3, 54) = 0.36, p = .78$ . Performance in a cache-recovery task could be affected by differences in spatial memory, activity levels, or motivation. The fact that control and nutritionally deprived birds cached similar amounts of food suggests that activity levels were not affected by our treatment.

It remains possible, however, that the differences between the experimental and control scrub jays in cache-recovery performance could be explained by differences in motivation. We addressed this issue in the experiments described below.

### Associative Learning Task

We tested memory performance of jays approximately 8 months old in an associative learning task in which birds had to remember a location in which they had found food previously. During five rewarded trials in a spatial version of the task, nutritionally deprived birds exhibited significantly lower memory performance than controls: repeated measures GLM,  $F(1, 18) = 13.95, p < .01$  (see Figure 4). Performance of both experimental and control groups did not vary significantly across the five rewarded trials: trial,  $F(4, 72) = 0.54, p = .71$ , and Trial  $\times$  Treatment,  $F(4, 72) = 1.54, p = .20$  (see Figure 4). Nutritionally deprived jays performed worse than controls in all five trials (in contrast analyses, all  $ps < .05$ ). However, both nutritionally deprived and control birds performed better than would be expected from a random search during all five trials (paired  $t$  test, all  $ps < .05$ ).

The differences among the groups remained significant in an unrewarded probe trial following the five rewarded trials; nutritionally deprived birds searched more sites than controls in order to find the site that had been associated with food during the five previous trials,  $t(17) = 2.24, p = .04$  (see Figure 4). Birds in both groups performed significantly better than would be expected from a random search ( $ps < .001$ ).

During five rewarded trials in a color version of an associative learning task, in which the rewarded site was uniquely color-

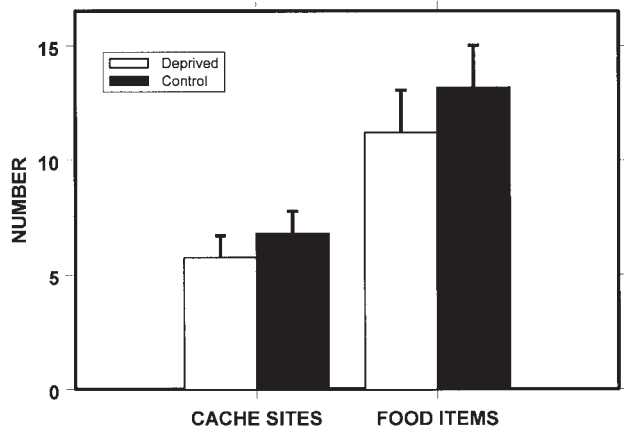


Figure 2. Mean number of cached food items and cache sites used by nutritionally deprived (open bars) and control (filled bars) jays in four cache-recovery trials.

marked and the birds could use both spatial and local (color) information to find food, memory performance of nutritionally deprived birds did not differ significantly from that of controls: repeated measures GLM,  $F(1, 18) = 3.37, p = .08$  (see Figure 5). The contrast analyses showed marginal differences among the groups during the first trial ( $p = .08$ ), but both groups' performance converged in consecutive trials ( $ps > .10$ ), as nutritionally deprived birds rapidly improved their performance. Overall, birds' performance varied among the trials: trial,  $F(4, 72) = 12.99, p < .001$ , and Trial  $\times$  Treatment,  $F(4, 72) = 1.26, p = .29$  (see Figure 5). However, only nutritionally deprived jays' performance showed significant improvements: Trials 1 and 2,  $p = .01$ ; Trials 4 and 5,  $p = .03$ ; performance of control birds did not vary significantly between the trials (contrast analyses,  $ps > .15$ ). Both nutritionally deprived and control jays performed better than

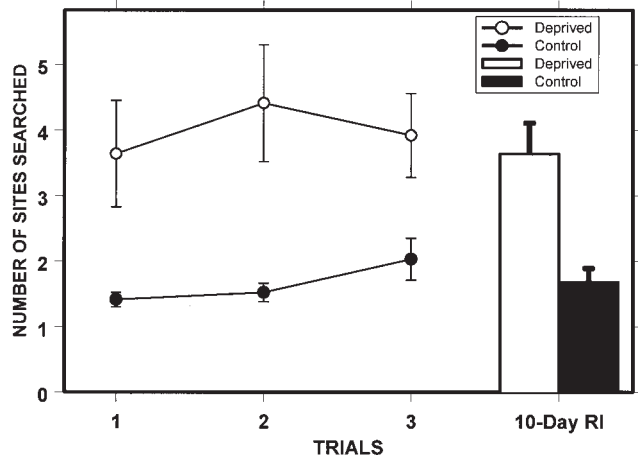


Figure 3. Memory performance in a cache-recovery task measured as the number of sites inspected by nutritionally deprived (empty circles and bars) and control (filled circles and bars) scrub jays in order to find previously hidden caches. Error bars represent standard errors. Trials 1-3 had a 1-day retention interval (RI), and the fourth trial had a 10-day RI.

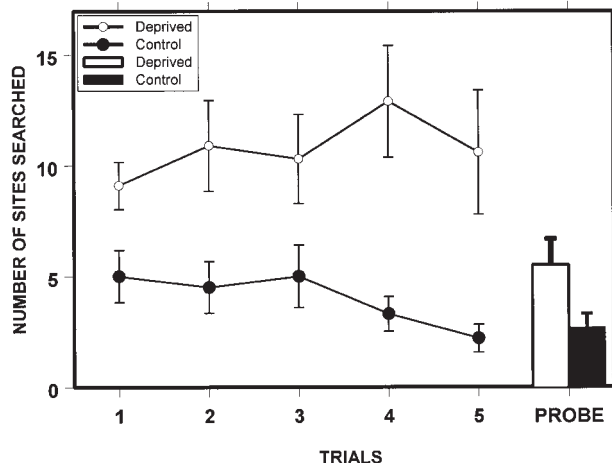


Figure 4. Memory performance in the spatial version of an associative learning task measured as the number of sites inspected by nutritionally deprived (empty circles and bars) and control (filled circles and bars) scrub jays in order to find the site previously containing food. Error bars represent standard errors. The first five trials contained Phase 1 (food visible) and Phase 2 (food hidden) separated by a 2-hr retention interval. Performance was measured during Phase 2, and food was present at the rewarded location. Food was at the same location for the five trials, which were run every 5th day. An unrewarded probe trial (bars; 5 days after the fifth rewarded trial) contained only Phase 2, and no food was present.

would be expected from a random search during all five trials (paired  $t$  test, all  $ps < .05$ ).

In an unrewarded dissociation probe trial, in which the color associated with the previously rewarded site was shifted to a new

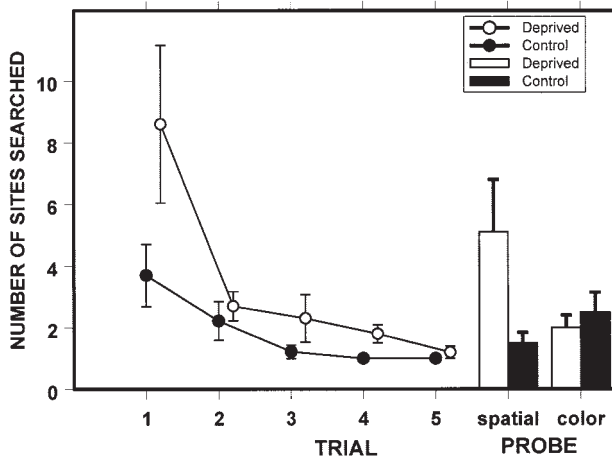


Figure 5. Memory performance in the first color version of an associative learning task measured as the number of sites inspected by nutritionally deprived (empty circles and bars) and control (filled circles and bars) scrub jays in order to find the site previously containing food. Error bars represent standard errors. The first five trials contained Phase 1 (food visible) and Phase 2 (food hidden), separated by a 2-hr retention interval. Performance was measured during Phase 2, and food was present at the rewarded location. Food was at the same location for the five trials, which were run every 5th day. Five days after the fifth rewarded trial, we ran an unrewarded color dissociation probe trial (bars), during which no food was present and the color was moved to a new location.

location, nutritionally deprived birds inspected more sites than controls in order to find the previously rewarded, unmarked site at the correct spatial location,  $t(18) = 2.51, p = .02$  (see Figure 5). In contrast, nutritionally deprived and control birds did not differ significantly in the number of searches made prior to inspecting the new color location,  $t(18) = -1.47, p = .16$  (see Figure 5), indicating that the differences between nutritionally deprived and control jays were specific to spatial memory. Birds in both groups performed better than would be expected from a random search at both the spatial and color locations ( $ps < .05$ ).

The probe trial allowed us to determine whether jays relied preferentially on spatial information (in which case we would expect them to search in the correct spatial location before searching in the new color location) or on color information (in which case we would expect birds to search in the new color location prior to searching the correct spatial location). The order in which individual birds inspected the spatial location or the new color location revealed significant differences between nutritionally deprived and control jays,  $\chi^2(1, N = 20) = 5.49, p = .02$ . Nine out of 10 nutritionally deprived jays inspected the new color location prior to inspecting the spatial location, irrespective of the total number of searches to find both locations (significantly more than expected by chance),  $\chi^2(1, N = 10) = 3.81, p = .05$ . In contrast, 6 control birds inspected the spatial location first, and 4 control jays inspected the new color location first,  $\chi^2(1, N = 10) = 0.20, p = .65$ .

In a second color version of an associative learning task in which birds had to learn the color associated with food irrespective of its spatial location, repeated measures GLM indicated that nutritionally deprived jays performed similarly to controls when choosing among five available colors during four rewarded trials,  $F(1, 18) = 2.81, p = .11$  (see Figure 6), as well as during the unrewarded probe trial,  $t(18) = 1.35, p = .19$  (see Figure 6). Both nutritionally deprived and control jays performed better than would be expected from a random search during all trials (paired  $t$  test, all  $ps < .05$ ). These results again suggest that memory for color was not affected by nutritional deficits during posthatching development.

### Brain Measurements

Compared with controls, 1-year-old nutritionally deprived scrub jays had significantly smaller (approximately 8%) relative hippocampal volume,  $F(1, 17) = 4.83, p = .04$  (see Figure 7A), and fewer (approximately 11%) hippocampal neurons,  $F(1, 17) = 8.95, p < .01$  (see Figure 7B). To establish whether nutritional deficits during early posthatching development affected overall brain growth, we also measured telencephalon volume and overall brain mass. The volume of the telencephalon (minus the hippocampus) was not affected by our treatment,  $F(1, 18) = 0.25, p = .62$ , and brain mass was also indistinguishable between experimental and control scrub jays,  $F(1, 18) = 0.20, p = .66$ . These findings indicate that nutritional deficits during early posthatching development influenced the morphological characteristics of the hippocampus and that nutritional rehabilitation from 2 months to 1 year of age did not alleviate the effects of early malnutrition.

### Discussion

Our experiment demonstrated that 1-year-old scrub jays that experienced nutritional deficits during early posthatching develop-

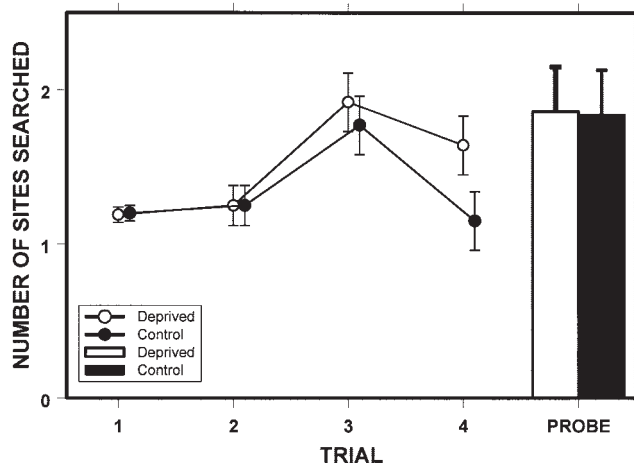
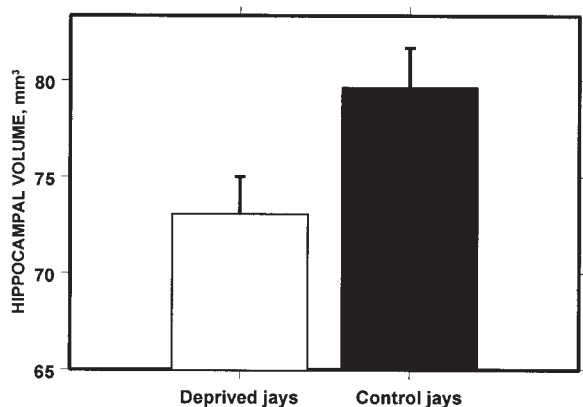


Figure 6. Memory performance in the second color version of an associative learning task measured as the number of colored sites (five different colors) inspected by nutritionally deprived (empty circles and bars) and control (filled circles and bars) scrub jays in order to find the color associated with food. Error bars represent standard errors. The first four trials contained Phase 1 (food visible) and Phase 2 (food hidden), separated by a 2-hr retention interval. Performance was measured during Phase 2, and food was present at the location associated with rewarded color. All five colors were associated with different locations during both phases and during the four rewarded trials, which were run every 5th day. Five days after the fourth rewarded trial, we ran an unrewarded probe trial (bars), during which no food was present, and all five colors were associated with uniquely new locations.

ment had smaller hippocampi with fewer neurons as compared with normally developing birds. Spatial information processing previously has been linked to hippocampal function in both birds and mammals (Sherry et al., 1992; Sherry & Vaccarino, 1989; Hampton & Shettleworth, 1996). In our study, nutritionally deprived birds with reduced hippocampal volume and fewer hippocampal neurons showed inferior performance in a cache-retrieval task and in a spatial version of an associative learning task, as compared with control jays. In contrast, nutritionally deprived scrub jays did not differ from controls in their performance on two color versions of an associative learning task, which is thought to be hippocampus-independent (Hampton & Shettleworth, 1996; Sherry & Vaccarino, 1989). Nutritional deficits did not influence overall brain growth because neither the telencephalon (minus the hippocampus) volume nor total brain mass was different between nutritionally deprived and control scrub jays.

In the hippocampus-independent associative learning task, when the spatial location was marked by a unique color, performance of nutritionally deprived jays improved to the level of controls, indicating that the motivation to search for food was similar for birds in both groups and that the differences observed in the cache-recovery task and the spatial version of the associative learning task were most likely due to differences in spatial memory. The fact that both groups performed better than would be expected from a random search on all trials also indicated that all birds relied on memory and were motivated to find the locations associated with food. Furthermore, all birds went directly to the site containing food during Phase 1 in all versions of the associative learning task, and there were no differences in food-caching ac-

A



B

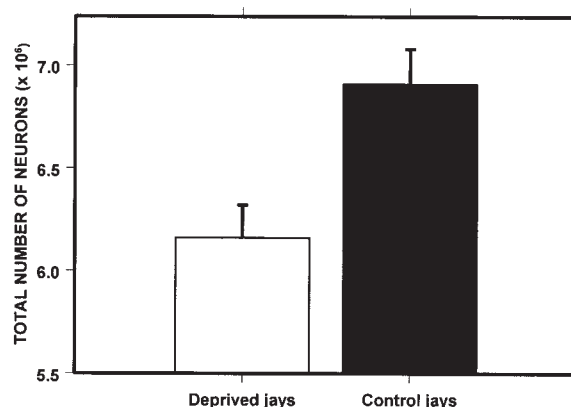


Figure 7. (A) Volume of the hippocampal formation (mean and standard error) and (B) the total number of hippocampal neurons (mean and standard error) in nutritionally deprived (empty bars) and control (black bars) scrub jays.

tivity between the groups, suggesting that motor behavior did not differ between nutritionally deprived and control jays.

The color dissociation probe trial provided further evidence that memory performance impairments suffered by nutritionally deprived jays were specific to spatial memory. First, nutritionally deprived birds inspected significantly more sites than controls prior to finding the correct spatial location, which was no longer associated with the local (color) cues. In contrast, there were no differences between nutritionally deprived and control individuals in the number of sites inspected prior to finding the new color location. Second, as compared with controls, more nutritionally deprived birds inspected the new color location prior to finding the correct spatial location; in fact only 1 of 10 experimental individuals checked the correct spatial location before going to the site associated with the local cues (color). It has been suggested that food-caching birds rely on spatial information rather than on local (color) information and thus that they should attend preferentially to spatial cues (Brodbeck, 1994). Our experiment demonstrated that this was not necessarily the case in either nutritionally deprived or control scrub jays; even among control jays, about half of the birds attended to color cues prior to inspecting the correct spatial location. Control birds seemed to rely equally on spatial and local (color) information to find locations associated with food, because individuals that inspected the new color location first, then immediately inspected the correct spatial location and vice versa. In contrast, whereas nutritionally deprived jays had no problems finding the new color location, they searched significantly more sites than controls in order to find the correct spatial location.

Finally, when jays had to learn that food was associated with one out of five colors irrespective of their spatial locations, there were no significant differences between nutritionally deprived and control birds, suggesting that memory for color was not affected by our treatment and that differences detected in a cache-retrieval task and in the spatial version of an associative learning task were specific to spatial memory.

Previously, several studies indicated that song learning in male birds might be impaired by nutritional stress during development and suggested that impaired song learning abilities provide an indirect signal of inferior quality of these males to their potential mates (Buchanan et al., 2003; Nowicki et al., 2002; Spencer et al., 2003). These studies suggested that song learning is an indicator that may correlate with some other phenotypic and/or genotypic variables that could directly affect fitness. It has also been shown that food deprivation during early posthatching development could result in smaller body size (Searcy et al., 2004) and in reduced parasite resistance (Buchanan et al., 2003), which could lead to higher mortality. Our study further supports the idea that nutrition during early posthatching development may have serious implications for future fitness by showing that nutritional deficits similar to what could be observed between siblings within natural broods during early posthatching development have long-term negative effects on the hippocampus and spatial memory, which could have a direct impact on future survival and fitness (Pravosudov & Lucas, 2001).

The differences in hippocampus structure and spatial memory performance between nutritionally deprived and control scrub jays were observed more than 7 months after achieving nutritional independence despite the fact that there were no differences in telencephalon volume or brain mass. These results indicate that nutritional rehabilitation (after the jays became independent and had unlimited access to food) did not alleviate the deleterious effects of malnutrition during the early posthatching period. These findings are particularly important with respect to the effects of malnutrition during development on brain growth, cognitive abilities, and general fitness later in life. From an evolutionary biology perspective, these memory impairments provide the critical link that might explain why slower growing individuals may suffer lower fitness and increased mortality later in life. Food-caching animals in particular should be strongly affected by memory impairments because their survival critically depends on the ability

to locate previously hidden caches (Pravosudov & Lucas, 2001), and it is well known that such animals use spatial memory to do so (Shettleworth, 1995).

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